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IMAGING VENATION OF BROADLEAF PLANTS USING DSLR CAMERAS, TRASMITTED LIGHT, AND DIGITAL FILTERS

DANIEL MCNAIR
Department of Biological Sciences
University of Southern Mississippi
Hattiesburg, Mississippi 39406
danielmcnair@gmail.com

ABSTRACT

Methods are outlined for obtaining high-quality digital images of venation in broadleaf plants using a DSLR camera, transmitted light, and digital filters (including edge detection filters). Mounted specimens can be imaged using an external flash placed beneath the specimen. This technique can be used on type specimens or rare collections when destructive sampling is not possible. Also outlined here is a common method used by botanists and nature photographers for using a handheld camera and transmitted sunlight to capture venation details in the field. Step-by-step instructions are given for these methods, and examples are provided with detailed notes on camera settings and digital filters. These techniques are not new, but neither are they often implemented in literature dealing with venation, likely because the standard protocol of chemically clearing and staining leaves provides the highest level of detail. However, the methods explained here usually show three or four orders of venation, which should prove useful in various taxonomic and ecological studies of broadleaf plants.

The Manual of Leaf Architecture (Ellis et al. 2009) makes a compelling case for the use of venation characters in taxonomic descriptions of broadleaf angiosperms, but there are also important paleoecological reasons to study venation. Boyce et al. (2009) show that among vascular plants, both modern angiosperms and fossil angiosperms have uniquely dense veins, a trait that allows higher transpiration rates and may help explain the modern dominance of angiosperms. Leaf veins are also informative in studies of plant physiology and development (for a brief review see Blonder et al. 2012). In the traditional method of studying venation, leaves are chemically cleared and stained, a type of preparation that is essential for observing the highest level of detail of most characters, especially under a microscope (see Vasco et al. 2014 for recent methods). But cleared leaf photographs are often left out of literature on modern plants such as guides to floras or descriptions of new species. Perhaps this has something to do with the time and materials involved in preparing the leaves or possibly the aesthetic challenges of photographing cleared specimens, since they have to be mounted in transparent material or floated in ethanol (Vasco et al. 2014). Additionally, the leaves of many species often break apart under chemical treatment (Blonder 2012).

When a leaf is backlit, the high transmission of visible light through the vascular tissues of leaves (i.e., the veins), creates contrast with the other tissues (Fig. 1). Nature photographers often take advantage of this property of leaves, but floristic treatments often do not. There are notable exceptions. For example, Brooker and Nicholle (2013) wrote a guide to the leaves of *Eucalyptus* and relatives using field images taken with transmitted sunlight. They explain that one of the benefits of photographing living leaves is that the oil cells in *Eucalyptus* relatives are obscured in dried specimens. Also, Harms (2015) has briefly explained how to use artificial transmitted light to photograph venation in the genus *Evolvulus*. Major herbaria, on the other hand, have imaged millions of plant specimens using *incident* lighting. This type of lighting probably gives a very true-to-life representation of most details, with the notable exception of venation (Fig. 1). Blonder et al. (2012) showed that x-raying leaves, a quick and relatively non-destructive method, can produce high quality

imagery of leaf venation, though at a cost that might be prohibitive for some projects. There has also been research on the use of edge detection filters and similar algorithms to extract venation information from leaf images photographed with incident light (Cope et al. 2010; Sanjeev et al. 2012).

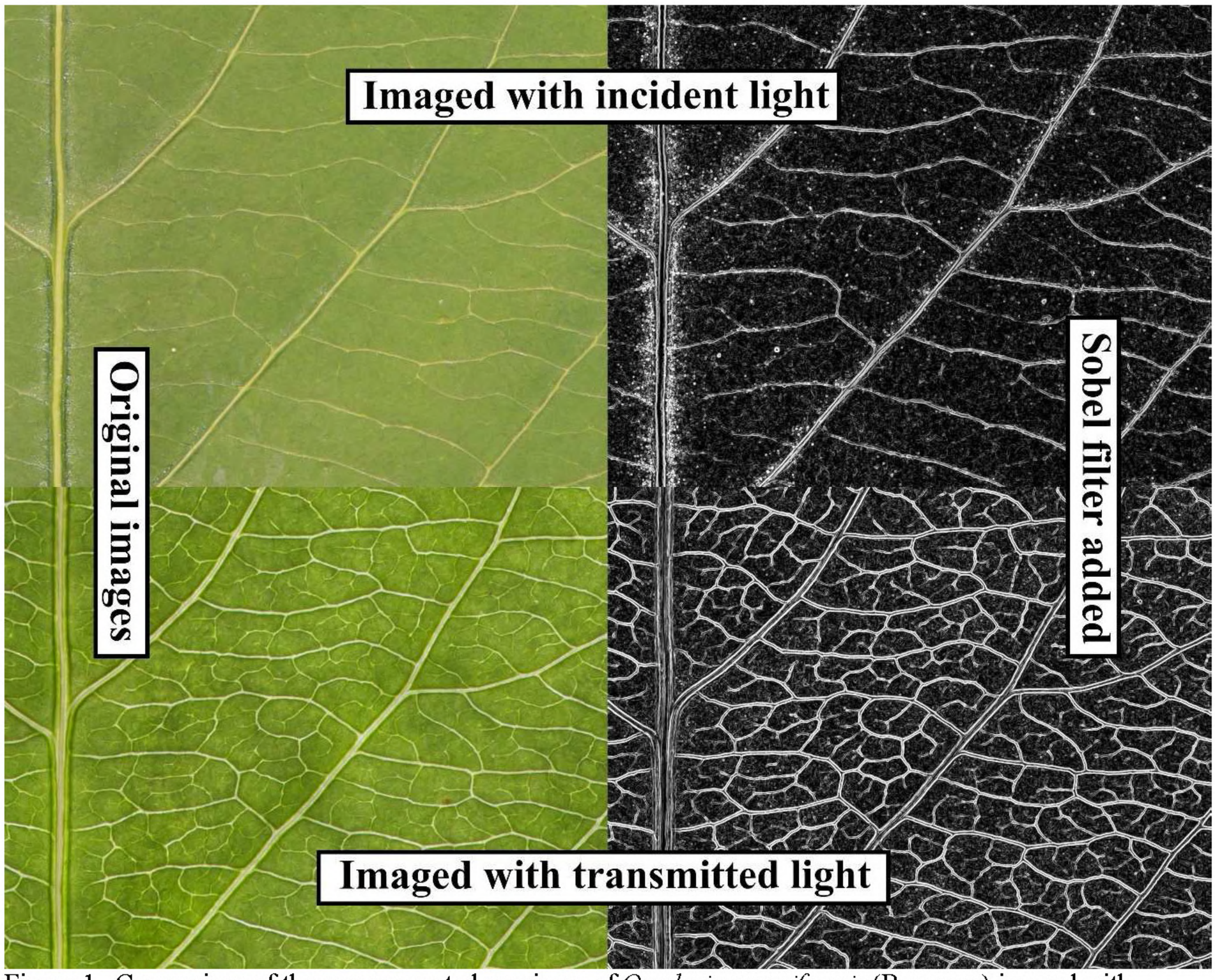

Figure 1. Comparison of the same mounted specimen of *Oemleria cerasiformis* (Rosaceae) imaged with incident light (top) and transmitted light (bottom). All camera settings and filters applied were identical for both sets of images. An external flash was used for both images. Sobel edge detection filters and grayscale applied in GIMP 2.8.

The methods described here are quick, non-destructive, and relatively inexpensive. In the examples provided (Figures 1, 4–10), three to four orders of veins are clearly discernable (sensu Ellis et al. 2009). These methods should not be considered a replacement for traditional clearing and staining methods but rather an alternative in situations when chemical treatment is impractical or impossible, as is the case with many type specimens or rare collections.

Equipment and techniques

The image quality of cropped-frame DSLRs works well for this type of imaging; however, full-frame sensor DSLRs usually have slightly better ISO to noise ratios. A macro lens is preferable, that is, a lens with a reproduction ratio greater than 1:1. The technique used for mounted specimens requires a tripod or herbarium imaging stand and an external flash or other strong lighting source that can be placed beneath the specimen. The external flash does not have to be able to meter with the camera; thus, a relatively inexpensive manual flash can be used. The light source should be strong

enough to pass through both the herbarium mounting paper and the leaf being photographed. Additionally, the side of the leaf specimen facing the camera can be important. All the examples given below feature the adaxial surface of the leaf facing the camera.

The images presented here as examples (Figures 1, 4–10) were taken using a Nikon D610 and Tokina 100 mm macro lens. Initial editing was done in Adobe Lightroom 5.6 and edge detection filters were applied in GIMP 2.8 (a free program) or Photoshop CC2014. Though the algorithm for Photoshop's Find Edges filter is not given, very similar results can be obtained by using GIMP's Differential filter set at 4 or 5 and with inverted colors. Edge detection filters can highlight some venation features and obscure others and so should be used with caution.

Technique for imaging mounted specimens using a tripod and artificial light (Figures 1–2, 4–8)

1. Create a platform that raises the mounted specimen approximately 0.3 m so that an external flash can be placed under the specimen. Place the external flash to one side of the leaf being photographed so that the illumination strikes the subject at approximately a 45° angle. This will help reduce glare from the flash. The position of the light source is very important and may require some experimentation depending on the equipment used. If light is concentrated too heavily in one area of the image, a diffuser may be needed. Mount the camera to a tripod, and position the camera directly over the specimen. A level may be required to align the camera with the specimen. Many herbaria are equipped with imaging stands that could be easily adapted to this method.
2. Switch camera to Manual mode and image quality to RAW.
3. Ideally, the ISO should be set as low as possible to reduce sensor noise (i.e., less than 400 on a full-frame camera and less than 100 on a cropped frame camera).
4. Shutter speed should be set somewhere between 1/20 and 1/150 (most cameras are not set up to exceed 1/200 while using a flash).
5. Aperture should be set depending on the lens, but 8 to 15 is usually a good range. Different lenses have different "sweet spots" of greater sharpness. An f-stop lower than 8, especially with a macro lens, will make it difficult to get the entire leaf plane in focus.
6. Perform test shots to find the right combination of shutter speed, aperture, and ISO.
7. Recommended: a bright incident light source such as a flashlight will help when focusing the lens manually.

Field photography technique with handheld camera and natural light (Figures 3, 9–10)

1. Set camera image size to RAW or highest resolution JPEG (if camera memory is an issue).
2. Set ISO from 100 to 1000, f-stop from 8 to 15, and shutter speed from 1/200 to 1/800. Optimal settings will depend on the amount of sunlight available.
3. Select a leaf specimen that is free of dirt and insect damage and that has a relatively flat plane.
4. Position the leaf with sunlight evenly back-lighting the area of interest. Also, position the subject so that an area directly behind it is in relative darkness (e.g., dense shrubs). Avoid allowing the light source to enter directly into the lens, as this will create a glare in the image.
5. Perform test shots and adjust camera settings (primarily shutter speed, aperture, and ISO) accordingly so that the leaf is properly exposed but the background is dark. The background does not have to be completely dark since shadows can be exaggerated, i.e., turned into blackness using digital filters.

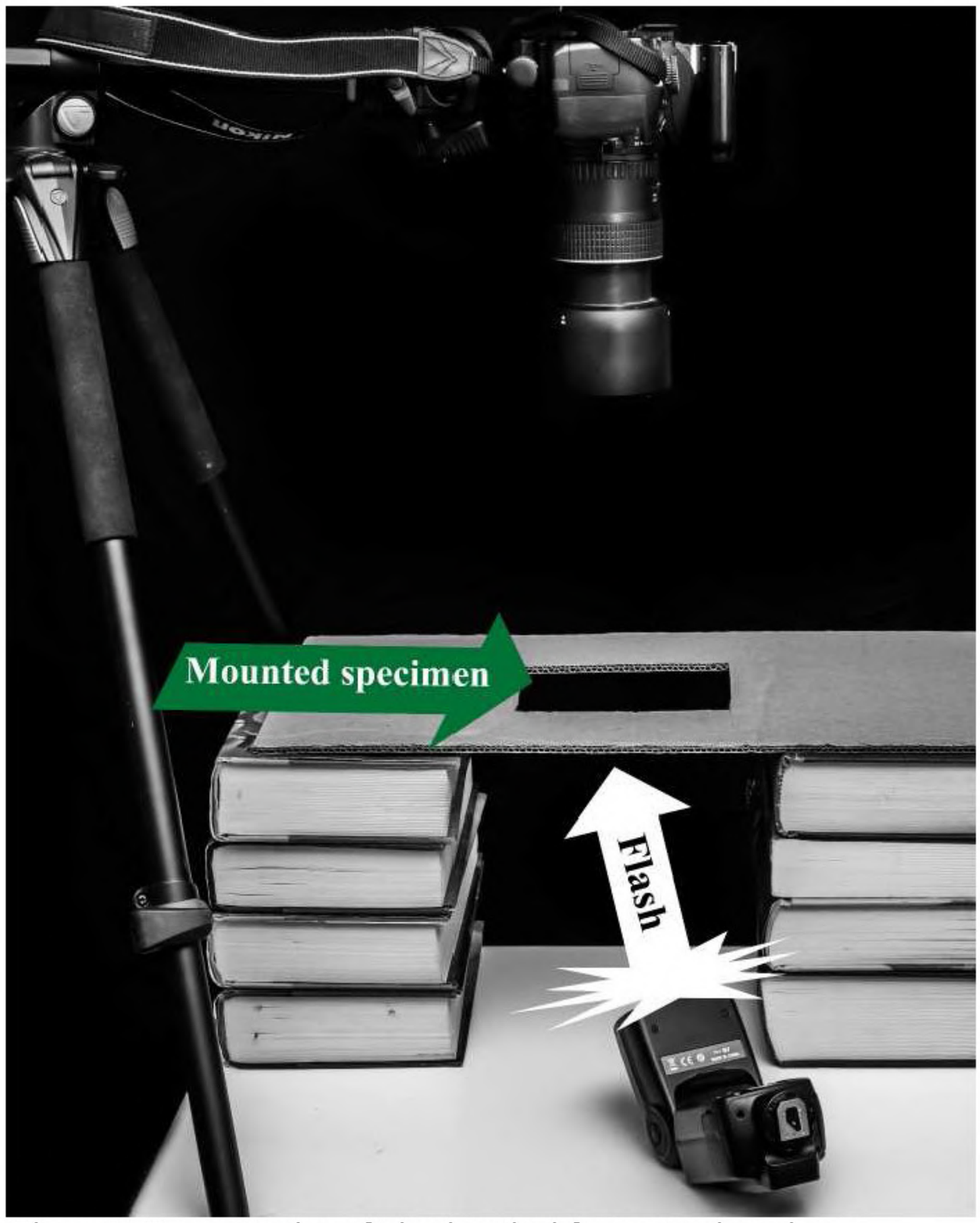

Figure 2. Demonstration of tripod method for mounted specimens.

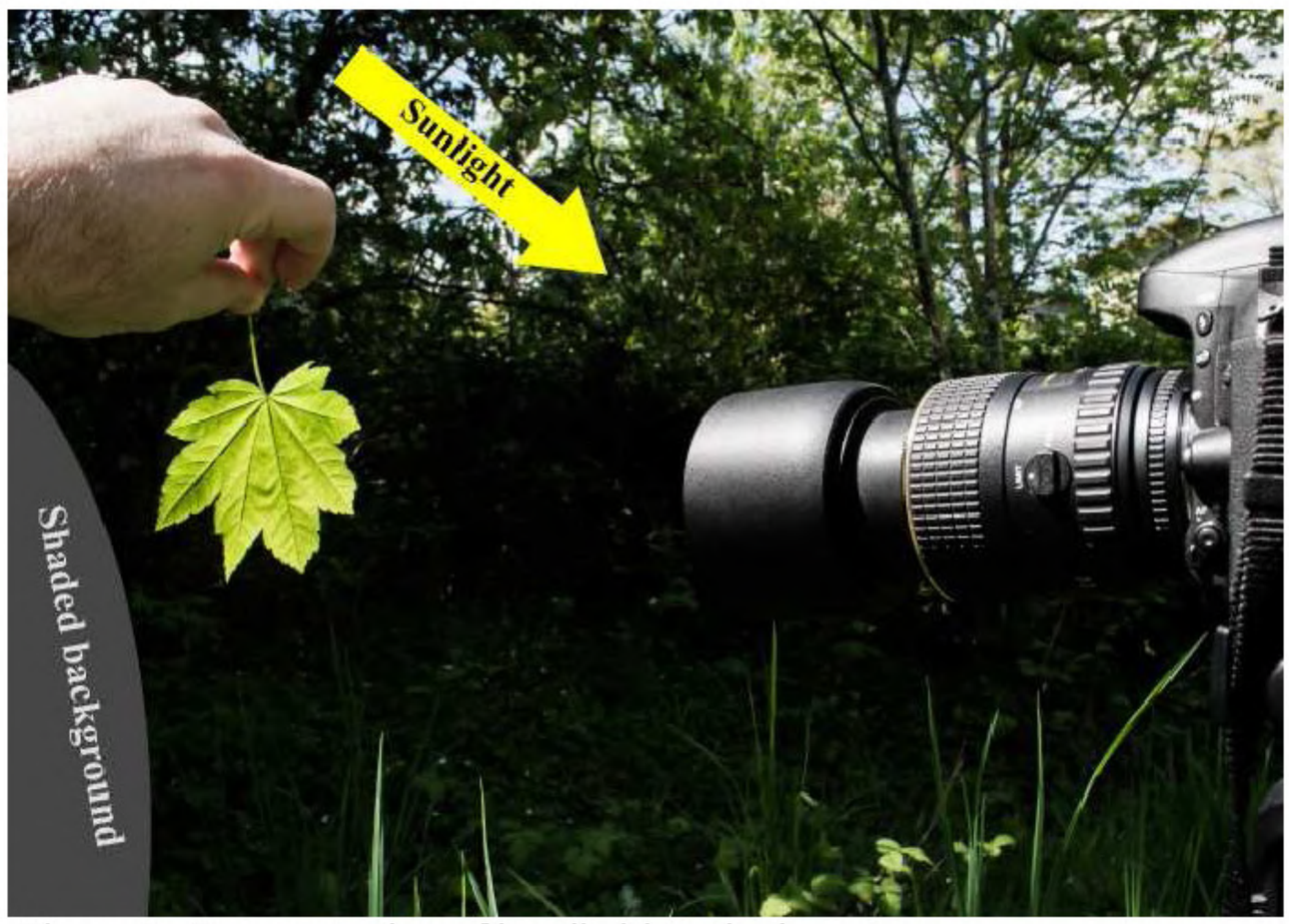

Figure 3. Demonstration of handheld method.

Image processing in Adobe Lightroom and GIMP (Figure 4)

1. Recommended: apply Black & White filter.
2. Generally, Contrast and Sharpness should be increased.
3. Apply a noise reduction filter (Luminance Smoothing), especially if cropping the image.
4. Adjust Exposure, Highlights, and Shadows so that venation is visible. Adjusting for a completely black or white background is not necessary but tends to hide noise artifacts in the background.
5. Use the manual Adjustment Brush tool to fix uneven exposure dark spots on the herbarium mounting paper. Photographs of living specimens may require more of these localized exposure adjustments since they are often more textured than pressed specimens.
6. Optional: apply a digital edge detection filter in Photoshop or GIMP.

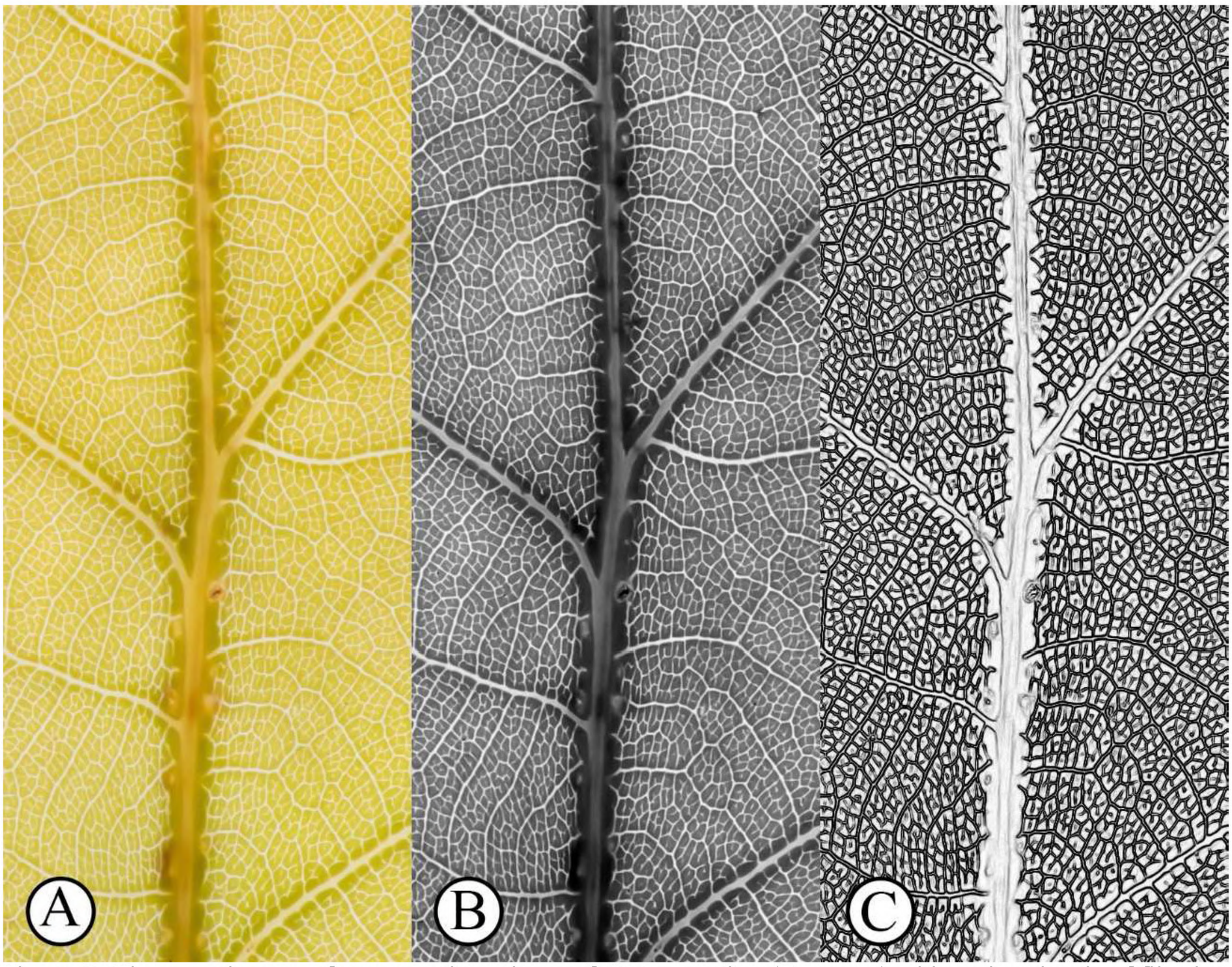

Figure 4. The same images of a mounted specimen of *Quercus robur* (Fagaceae) with various levels of filtering applied. All the images have noise reduction added in Lightroom. **A.** Unedited image, i.e., as imported into Lightroom. **B.** Edited in Lightroom (Black & White, Contrast +60, Shadows +80, highlights -50, Clarity +50.) **C.** Image from previous with Find Edges added in Photoshop.

Figure 5. Mounted specimen of *Amelanchier alnifolia* (Rosaceae) photographed with Nikon D610, Tokina 100 mm lens, tripod, and Yongnuo 560 III speedlight at 1/2 illumination. ISO 200, f/11, 1/50 s. Edited in Adobe Lightroom 5.6 (Black & White, Exposure + 1.243, Contrast + 100, Highlights +55, Shadows -26, Whites +45, Clarity +71, Luminance Smoothing 39).

Figure 6. Mounted specimen of *Holodiscus discolor* (Rosaceae) photographed with Nikon D610, Tokina 100 mm lens, tripod, and Yongnuo 560 III speedlight at 1/2 illumination. ISO 320, f/9, 1/40 s. Edited in Adobe Lightroom 5.6 (Black & White, Exposure +1.07, Contrast +95, Highlights +90, Shadows -20, Whites +100, Blacks -43. Clarity +30, Luminance Smoothing 48).

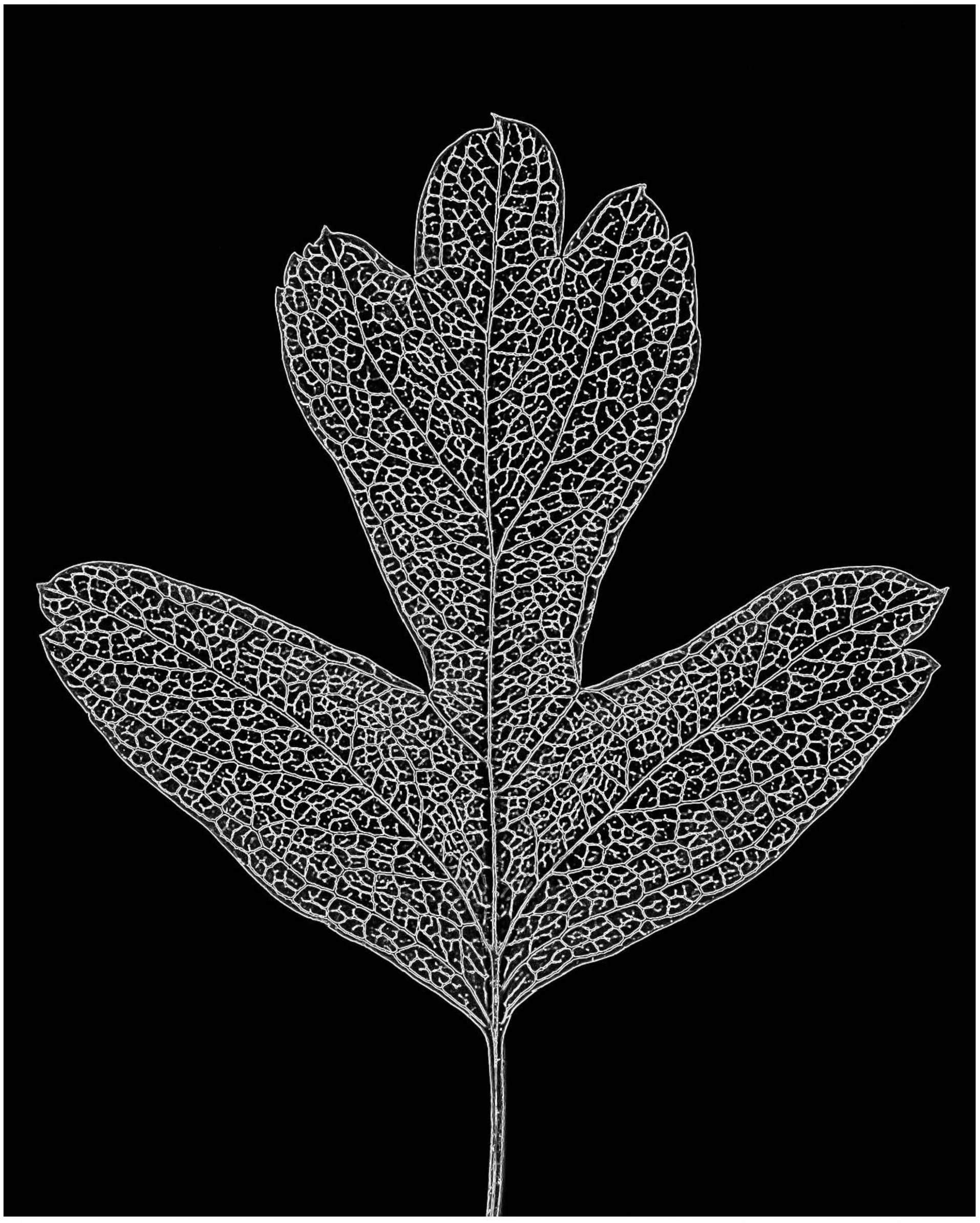

Figure 7. Mounted specimen of *Crataegus monogyna* (Rosaceae) photographed with Nikon D610, Tokina 100 mm lens, tripod, and Yongnuo 560 III speedlight at 1/2 illumination. ISO 320, f/9, 1/40 s. Edited in Adobe Lightroom 5.6 (Black & White, Exposure +1.9, Contrast +74, Highlights +50, Shadows -10, Whites +57, Blacks -60, Clarity +62, Luminance Smoothing 48). Sobel filter at setting 1 added in GIMP 2.8.

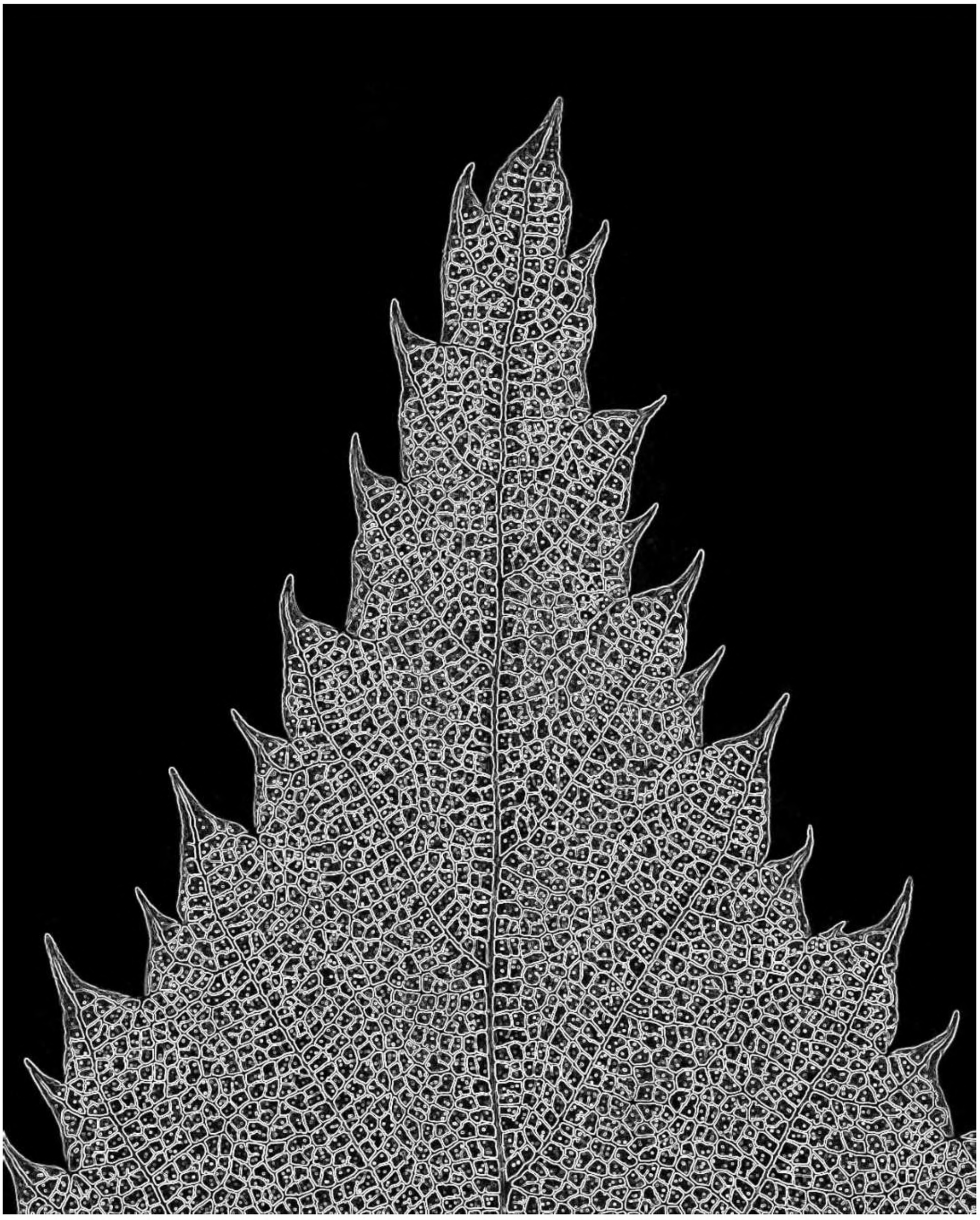

Figure 8. Mounted specimen of *Carpinus turczaninovii* (Betulaceae) photographed with Nikon D610, Tokina 100 mm lens, tripod, and Yongnuo 560 III speedlight at 1/2 illumination. ISO 320, f/14, 1/60 s. Edited in Adobe Lightroom 5.6 (Black & White, Exposure -1.2, Contrast +100, Highlights +12, Shadows +86, Whites 0, Blacks -60, Clarity +26, Luminance Smoothing 70). Sobel filter at setting 1 added in GIMP 2.8.

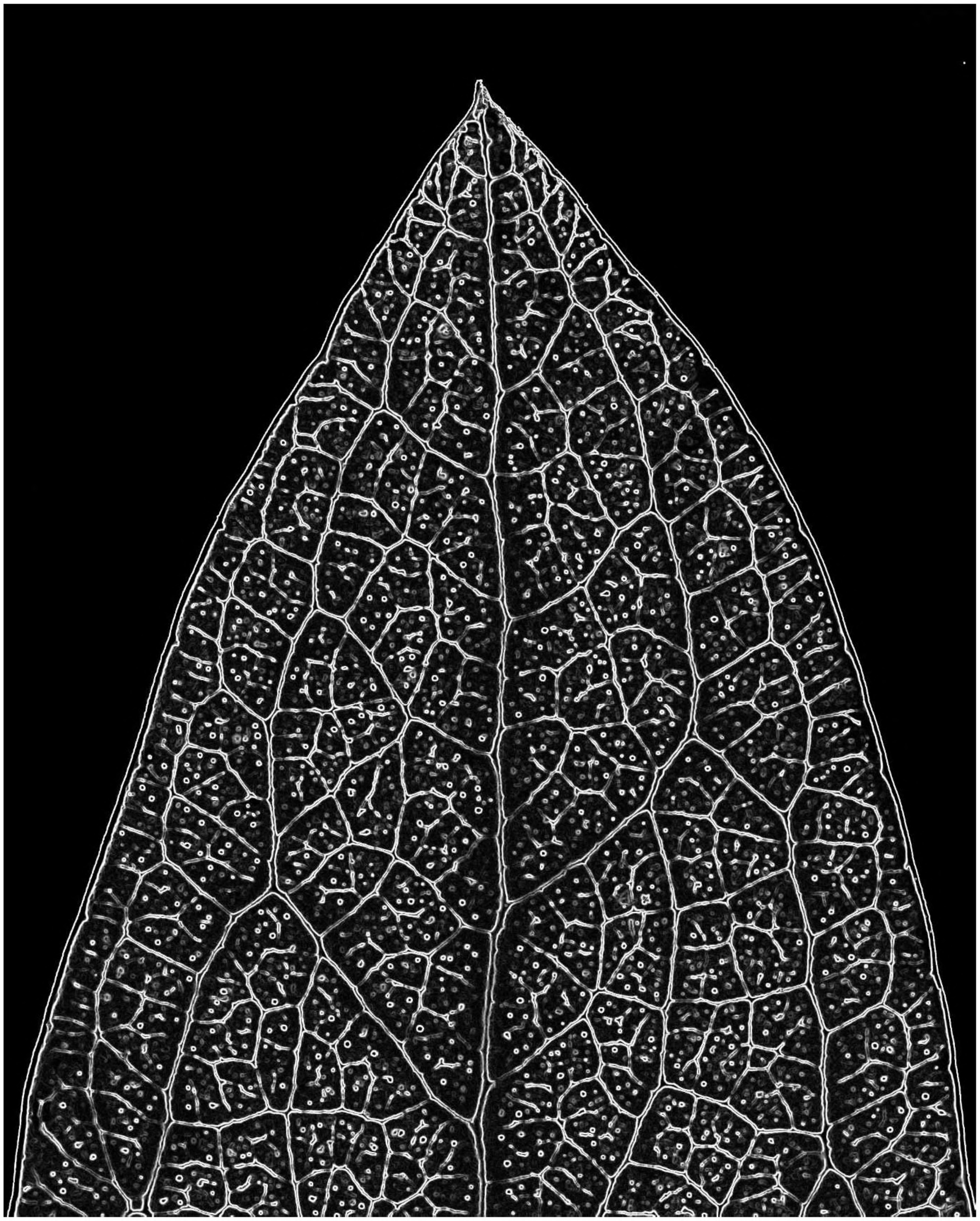

Figure 9. Living specimen of *Eucalyptus pauciflora* (Myrtaceae) photographed with handheld Nikon D610, Tokina 100 mm lens, and transmitted sunlight. ISO 1600, f/10, 1/640 s. Edited in Adobe Lightroom 5.6 (Black & White, Exposure -1.2, Contrast +100, Highlights +12, Shadows +86, Whites 0, Blacks -60, Clarity +26, Luminance Smoothing 70). Sobel filter at setting 1 added in GIMP 2.8.

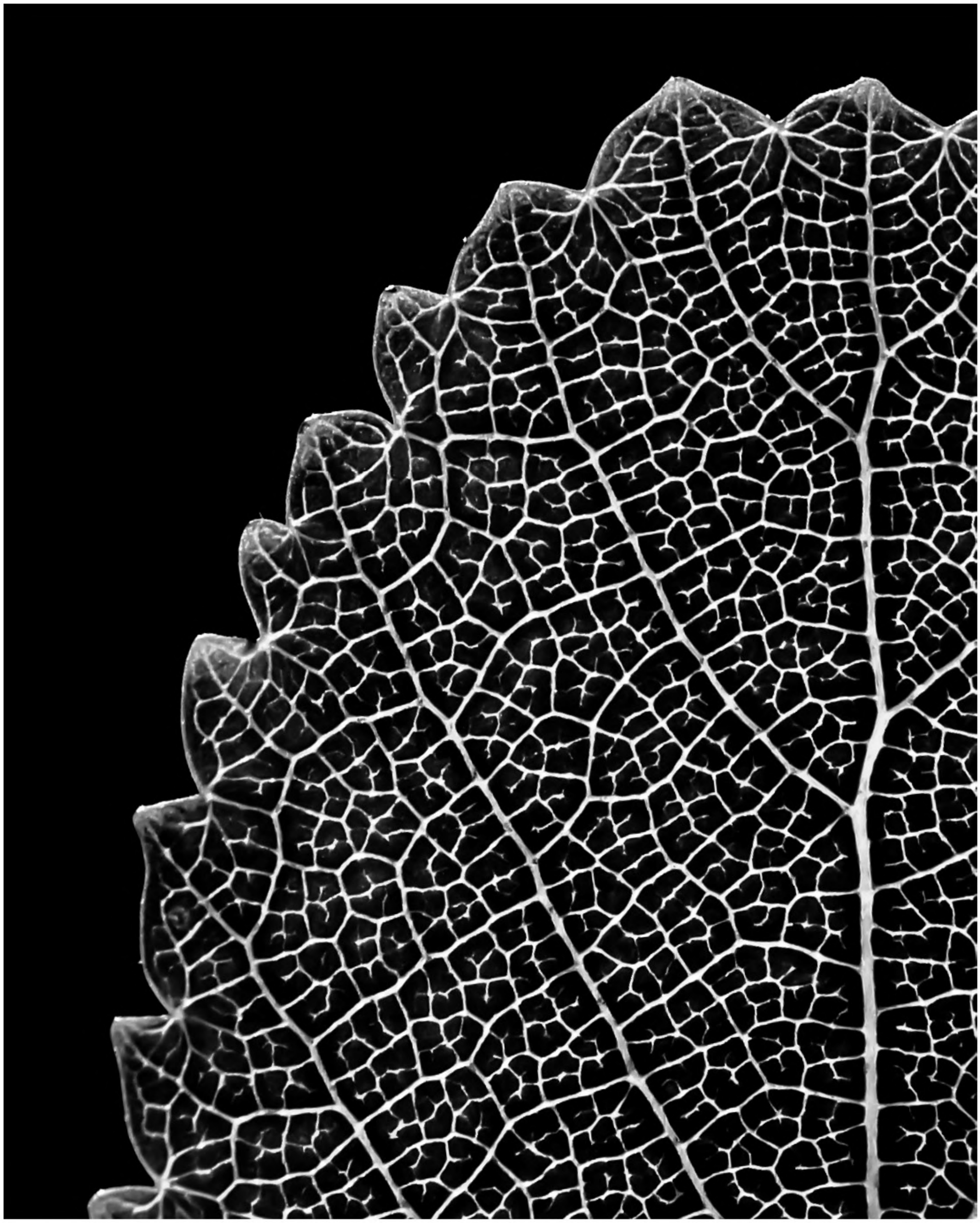

Figure 10. Living specimen of *Betula glandulosa* (Betulaceae) photographed with handheld Nikon D610, Tokina 100 mm lens, and transmitted sunlight. ISO 800, f/10, 1/800 s. Edited in Adobe Lightroom 5.6 (Black & White, Exposure -4.88, Contrast +38, Highlights -29, Shadows +29, Whites +74, Blacks -57, Clarity +81, Luminance Smoothing 73).

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